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# Considerations in the Design of an Environmental Specimen Bank: Experiences of the National Biomonitoring Specimen Bank Program

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Since 1979 the National Institute of Standards and Technology (NIST) has been involved in environmental specimen banking activities as part of the National Biomonitoring Specimen Bank (NBSB). These activities have focused on the development of procedures for the collection, processing, analysis, and long-term storage of a variety of environmental specimens including: human liver, mussels and oysters, fish tissue (liver and muscle), marine mammal tissues (blubber, liver, and kidney), and marine sediments. The experiences of the NBSB can provide valuable information to assist in the design of new specimen bank efforts. Based on the experiences of the NBSB, the issues that should be addressed in the design and operation of a valid specimen bank program are presented. — *Environ Health Perspect* 103(Suppl 3):61–67 (1995)

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## Introduction

During the past decade, specimen banking has been recognized as an important complement to traditional real-time environmental monitoring. The availability of well-documented and preserved environmental specimens provides a valuable resource for retrospective analysis as analytical techniques improve or as concerns about as-yet unidentified pollutants arise. In 1979 the National Institute of Standards and Technology (NIST), in conjunction with the U.S. Environmental Protection Agency (U.S.

EPA), established a pilot Environmental Specimen Bank Program to determine the feasibility of long-term storage of environmental samples (1,2). Human liver specimens were selected as the first sample type in this pilot program. Over 630 human liver specimens have been collected and archived as part of this program. Since 1985 the specimen banking activities at NIST have expanded to include marine sediments, mussels, oysters, and fish tissue collected for the National Status and Trends Marine Monitoring Program of the National Oceanic and Atmospheric Administration (NOAA); marine mammal tissue specimens from the Alaska Marine Mammal Tissue Archival Project and the National Marine Mammal Tissue Bank sponsored by Minerals Management Service (Department of the Interior) and NOAA, respectively; and total human diet specimens from a joint project of the U.S. Department of Agriculture (USDA), Food and Drug Administration (FDA), and the International Atomic Energy Agency (IAEA). These different specimen banking projects at NIST are known collectively as the National Biomonitoring Specimen Bank (3–5).

Other specimen banking programs have been established in Germany, Canada, Japan, and Sweden; and additional projects are in the planning stages (6,7). As current specimen banking activities expand and as new projects develop, these activities need

to be carefully designed to best fulfill the anticipated (and unanticipated) uses of these historical specimens. In this paper the experiences of the specimen banking activities at NIST will be described briefly with particular emphasis on the collection, storage, and analysis of human liver specimens. The issues that should be addressed in the design and operation of a valid specimen banking program are described.

## Current NIST Specimen Banking Activities

Since the establishment of the pilot Environmental Specimen Bank project in 1979 (1,2), specimen banking activities at NIST have expanded to include the banking of a variety of specimens from several projects of national and international interest. The National Biomonitoring Specimen Bank (NBSB) at NIST now consists of four major projects supported by various government agencies. The projects, and the year each was initiated are listed below. Then the current status of each project is briefly described

- EPA human liver specimen bank (1979)
- NOAA national status and trends specimen bank (1985)
- Marine mammal tissue bank projects including
  - MMS/NOAA Alaska marine mammal tissue archival project (1987)

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- NOAA national marine mammal tissue bank (1990)
- IAEA/FDA/USDA trace nutrients in human diet (1986)

The NBSB has provided a wide range of experience in the collection, processing, long-term storage, and analysis of specimen types including human liver, sediment, mussels, oysters, fish tissues (liver and muscle), marine mammal tissues (blubber, kidney, and liver), and total human diet specimens. A current inventory of the specimens included in the NBSB is provided in Table 1.

### U.S. EPA Human Liver Project

The purpose of the U.S. EPA Pilot Environmental Specimen Bank Project was to investigate the feasibility of specimen banking and to gain experience in the collection, processing, and long-term storage of environmental specimens for the determination of inorganic and organic constituents. The approach was designed to gain experience in the collection, storage, and analysis of four types of environmental accumulators (human, marine, food, and

air). However, since other government agencies involved in projects related to the marine environment and food specimens have joined the NBSB program, the U.S. EPA pilot specimen bank project has focused primarily on the continued collection of human liver specimens and research related to specimen banking. The goals of the U.S. EPA human liver project were to develop procedures for the collection, processing, and long-term storage of biologic specimens; to improve analytical methods for the determination of inorganic/organic contaminants in human tissue; to evaluate the long-term storage stability of biologic specimens; and to provide an archive of well-documented specimens of human liver for retrospective studies to determine long-term pollution trends and document the appearance of new pollutants. The U.S. EPA human liver project was designed as a pilot project to determine the feasibility of banking and not specifically as a monitoring program or for epidemiologic research.

At present, 632 human liver specimens in the NBSB have been collected since 1980 and archived as part of this project. Of these 632 specimens, 336 were collected during 1980 and 1981 in Seattle, Washington, Rochester, Minnesota, and Baltimore Maryland; 200 were collected during 1982 to 1989 in Seattle; and 96 have been collected since 1990 in Baltimore. We are currently collecting specimens from Baltimore at the rate of approximately 25 per year. Of the 632 liver specimens in the bank, 116 have been analyzed for approximately 30 trace elements and approximately 15 polychlorinated biphenyl congeners and chlorinated pesticides. This archive of human liver specimens represents a substantial resource for the sponsoring agency, U.S. EPA, and other research projects for the investigation of environmental pollution trends in human populations.

### National Status and Trends Specimen Bank

In 1985 NOAA incorporated specimen banking into the National Status and Trends (NS&T) Marine Monitoring Program. The NS&T program is a national monitoring program designed to quantify the current status and long-term trends in the concentration of selected contaminants and biologic indicators of contaminant effects in United States coastal and estuarine environments (8,9). The NS&T program consists of two separate monitoring activities: the Benthic Surveillance Project, in which sediment and fish tissues (muscle

and liver) are collected from approximately 80 coastal sites (40 sites per year); and the Mussel Watch Project, in which sediment and bivalve molluscs (mussels and oysters) are collected from over 200 coastal sites. During the first six years of the NS&T program, samples of sediment, mussels, and fish tissue were collected specifically for specimen banking from approximately 15 to 20% of the sites during the regular sample collection in both the Mussel Watch and Benthic Surveillance Projects. In addition to the regular collections for the NS&T specimen bank project, a number of specimens were collected in the Prince William Sound, Alaska, following the Exxon Valdez oil spill (1989) to assist in the assessment of the impact of this spill on the environment (Table 1). In 1992, samples of mussels collected from 82 sites between 1976 and 1978 as part of the EPA Mussel Watch Program were transferred to NOAA and relocated in the NBSB. These mussel samples had been stored at  $-20^{\circ}\text{C}$  since collection. At the NBSB the mussel tissue was removed from the shells and is now maintained in liquid nitrogen vapor freezers at  $-150^{\circ}\text{C}$ . Selected samples have been cryogenically homogenized and distributed for analysis at NIST and other laboratories. Even though these samples were not collected and stored under the rigorous protocols used for samples from the current NS&T program, they represent a valuable historical collection for comparison with samples from the same sites collected over 15 years later.

### Marine Mammal Tissue Bank Projects

**Alaska Marine Mammal Tissue Archival Project (AMMTAP).** The goal of the AMMTAP, which was established in 1987, is to archive a representative collection of Alaskan marine mammal tissues for future contaminant analyses and for documentation of long-term trends in environmental quality (10–13). In the past 7 years, selected tissues (blubber, kidney, and liver) from 91 individual marine mammals (nine species) from seven different sites in Alaska have been included in the bank. These specimens are obtained almost exclusively from animals killed during native subsistence hunts. More detailed discussions of this project, including the rationale for specimen selection, sample collection protocols, and a complete specimen inventory have been published (10–13).

#### National Marine Mammal Tissue Bank

In 1989 the Office of Protected Resources, National Marine Fisheries Service (NMFS) within NOAA initiated the development of

**Table 1.** Inventory of specimens in the National Biomonitoring Specimen Bank.

Human specimens	
Liver (U.S. EPA): 632 specimens	
Marine specimens	
Mussels (U.S. EPA)	
93 batches (70/batch) from one site	
82 batches from 82 sites of the 1976–1978 U.S. EPA Mussel Watch Program	
Benthic Surveillance Project (NOAA)	
Fish liver and muscle: samples from 33 sites	
Sediments: samples from 34 sites	
Mussel Watch Project (NOAA)	
Mussels and oysters: samples from 206 sites	
Sediments: samples from 163 sites	
Exxon Valdez oil spill damage assessment (NOAA)	
Fish muscle, liver, and sediments (4 sites)	
Mussels (17 sites)	
Alaska marine mammal tissue archival project (MMS/NOAA)	
Northern fur seal	15 animals
Ringed seal	32 animals
Bearded seal	5 animals
Harbor seal	3 animals
Spotted seal	1 animal
Stellar sea lion	1 animal
Walrus	2 animals
Belukha whale	15 animals
Bowhead whale	17 animals
National Marine Mammal Tissue Bank (NOAA)	
Harbor porpoise	10 animals
Pilot whale	9 animals
White-sided dolphin	4 animals
California sea lion	1 animal
Food specimens	
Total human diet (IAEA/FDA/USDA)	
8 collections	

a National Marine Mammal Tissue Bank patterned after the successful banking program for Alaska marine mammals. In 1990 a demonstration project was established in the northeast United States to evaluate the practical aspects of obtaining suitable specimens for the NMMTB from both incidental catches during commercial fishing and from mass strandings. In 1992 the NMMTB became part of a comprehensive marine mammal program within NOAA that also includes stranding networks, contaminant monitoring, and quality assurance of chemical analyses. This program is known as the National Marine Mammal Health and Stranding Network (NMMH&SN) program (14). NIST is involved in both specimen banking and analytical measurement quality assurance associated with the NMMH&SN program. A detailed discussion of the current status of the NMMTB has been reported by Lillestolen et al. (15). The AMMTAP is now coordinated with the NMMTB and both are managed by the Office of Protected Resources within NOAA. The NMMTB currently contains blubber and liver tissues from 24 animals (pilot whales, harbor porpoises, white-sided dolphins, and California sea lion).

#### **IAEA/USDA/FDA Nutrients in Human Diet**

The Nutrients in Human Diet Project is a joint program of several agencies to obtain comparative data on dietary intakes of nutritionally important minor and trace elements in a number of countries (16). As part of this effort, samples of the total human diet composites, collected as part of the FDA "market basket" survey (17) have been banked for long-term storage in the NBSB.

#### **Considerations in Design of a Specimen Bank**

Based on the experiences of the National Biomonitoring Specimen Bank (NBSB) projects during the past 14 years, a number of items should be considered in the design of a specimen bank program. These include the relationship of the specimen banking effort to an environmental/health monitoring program; specimen selection criteria and sample size; composite vs. individual specimens; collection, processing, and storage procedures; analysis of selected specimens; storage conditions; and sample access policy. Each of these items will be discussed relative to the experiences of the various projects within the NBSB.

#### **Specimen Banking as an Integral Part of a Monitoring Program**

Specimen banking offers an important complement to traditional environmental monitoring by providing the opportunity for retrospective analyses of specimens. Thus, specimen banking should be considered an essential part of any environmental/health monitoring program; and, ideally, the banking of specimens should be associated with an ongoing (or developing) monitoring program. Within the NBSB program, the NOAA NS&T Specimen Bank project and the two marine mammal projects represent activities associated with ongoing monitoring programs, whereas the U.S. EPA Human Liver project has not been associated with a monitoring activity. The following points should be considered in designing a specimen banking activity associated with an ongoing or proposed monitoring effort.

***Samples for Specimen Banking Should Be Collected at the Same Sites and Time as Samples Collected for the Monitoring Program.*** Coordination of these collection activities results in reduced costs and provides a direct link between the analytical data generated for both purposes. In the NS&T Specimen Bank program (9) and the AMMTAP/ NMMTB programs (14,15), samples are collected at the same time and place for both banking and monitoring. For example, in the marine mammal sampling projects, samples of blubber and liver tissue are collected from the same animals for both programs. Collection protocols for the samples intended for monitoring and banking may differ somewhat, i.e., the banking protocols are generally more stringent with regard to considerations of potential contamination and storage containers (see discussion below). In both of the marine mammal projects, the specimen banking activities were initiated first and the monitoring efforts were developed later.

***Specimens Should Be Specifically Collected for Banking.*** The long-term banking of specimens should not be an afterthought or be contingent on sample material being leftover from the samples intended for the monitoring program. Since the collection and processing of samples for long-term banking may differ from that of the monitoring program, specimens for each purpose should be collected using the required protocols.

***A "Representative" Number of Specimens from the Monitoring Program Should Be Banked.*** In environmental monitoring programs, large numbers (often hundreds) of samples are collected

and analyzed. However, not every specimen analyzed as part of the monitoring needs to be archived in the specimen bank. A specimen bank is not intended to provide comprehensive coverage of all the samples required for a monitoring program. Instead, the specimen bank should contain a "representative" number of specimens as defined by the anticipated future uses of the specimens and the storage space constraints. For example, during the first 6 years of the NOAA NS&T specimen bank project, samples were collected from about 30 Mussel Watch sites and 3 to 5 Benthic Surveillance sites per year specifically for archiving in the specimen bank. At this rate it was intended that after 6 to 7 years specimens would be included in the bank from almost all of the NS&T sites. At present, the NS&T specimen bank contains samples from 36 Benthic Surveillance sites and from 206 Mussel Watch sites. Collection of specimens from the NS&T program has been discontinued; however, the focus of any future banking efforts in this project will be to obtain specimens from sites that have not been included previously in the bank and to repeat collections at previous sites to provide banked samples collected at approximately 7- to 10-year intervals to allow long-term trend monitoring.

#### **Specimen Selection Criteria, Sample Size, and Number of Specimens**

In environmental monitoring programs, a wide variety of specimens has been used. However, in the selection of specimens for all banking activity within the NBSB, the following general guidelines have been followed in the selection of tissue types for the human and marine mammal projects:

- a minimum of two 150-g samples can be obtained (see below);
- the sample is conducive to precise anatomical description to allow consistency in sampling;
- the tissue provides a homogeneous sample;
- the tissue is readily accessible to sampling techniques;
- the tissue has the potential for concentrating both inorganic and organic contaminants (see below).

These guidelines were followed for the selection of liver as a target specimen for the U.S. EPA human tissue specimen banking program (1,2) and in both the AMMTAP and the NMMTB (10).

Both the U.S. EPA human liver project and the marine mammal tissue banking projects were designed specifically as

banking rather than monitoring projects. In the case of the marine mammal projects, the banking component was established first and the monitoring aspects were incorporated later. For the NS&T specimen banking project, the selection of specimens for banking was based on the earlier selection of the specimen types to be used for the monitoring program (mussels, sediment, and fish muscle and liver tissue).

**Storage Space Limitations Must Be Considered.** Space and cost constraints will limit the number and sizes of the samples stored in a specimen bank. Generally, it is not feasible to bank specimens corresponding to every sample type and sampling site from a monitoring program. Therefore, the selection of the appropriate specimens for archival storage is critical.

**A Sufficient Amount of each Specimen Should Be Banked to Allow Multiple Analyses over a Long Period.** A specimen bank is not just a temporary repository for samples from the monitoring program until analyses are to be performed. The banking of material for only one to two analyses does not constitute a specimen bank but rather temporary storage prior to analysis. Subsamples of archived specimens should be available for multiple analyses, e.g., for different studies, using multiple techniques focusing on different analytes, etc. In the NBSB projects, duplicate samples of approximately 150 g each are typically collected, thereby providing sufficient material for multiple analyses of the sample.

**Specimens (Tissues) that Accumulate Pollutants or Analytes of Interest Should Be Collected.** The most appropriate specimen type for a real-time monitoring program may not be the best specimen for long-term banking. Because of space limitations, specimens that accumulate pollutants or the analytes of interest are best suited for banking. In the U.S. EPA pilot project, one of the primary reasons for the selection of human liver as a representative human tissue for specimen banking was the fact that it accumulates both inorganic and organic contaminants. Human specimens such as blood or urine are more readily available and are generally used in human monitoring programs. However, these fluids reflect recent exposure rather than long-term contaminant accumulation. In addition, since the levels of environmental contaminants in these fluids are typically very low, large quantities of sample would be required to provide sufficient material for a banking program.

**Two Equivalent Samples Should Be Collected, One Available for Analysis and One for More Permanent Archiving.**

In all of the NBSB projects, one of the duplicate samples is available for homogenization to provide subsamples (typically 5 to 8 g each) for analyses (not analyses associated with the monitoring program, but analyses for specimen banking purposes as described below), whereas the second sample is intended for more permanent storage and should only be homogenized after all of the first specimen has been depleted (see sample access policy section). The second sample also represents a less processed specimen in that it has not been through homogenization or subsampling process that may potentially introduce contamination or cause degradation. Having two equivalent samples also provides a measure of security since the two samples are stored in different freezers.

**Composite vs Individual Specimens**

The decision on whether to bank specimens representing individual organisms or animals or a composite of a number of organisms or animals depends on the goals of the banking program. Is the goal to provide information, on the health status of individual organisms or animals or on selected populations (e.g., from a particular geographic area)? In the NBSB human liver and marine mammal tissue projects, the banked specimens represent individuals. In the NS&T project, composites of fish tissue from 50 to 200 fish or 30 to 50 mussels from a particular site are composited to provide the specimen for banking since the interest is in the contaminant status of an area, not in individual fish or mussels. Composite specimens may be necessary when insufficient material for banking purposes (see above) is available from individual organisms or animals.

**Collection, Processing, and Storage Procedures**

**Procedures Should Be Standardized and Well Documented.** Detailed sample collection protocols have been developed and implemented for each of the specimen types in the various projects, i.e., human liver (1); sediments, fish tissue, and mussels/oysters (9); and marine mammal tissues (10,11). The basic philosophy for these protocols focuses on the collection of duplicate samples; the use of collection and storage materials that minimize potential contamination; and freezing the sample as soon as possible after collection. A sample amount of about 150 g was selected as a

sufficient quantity to allow multiple analyses over a long period. The collection of two equivalent samples of 150 g provides one specimen for analyses and one for more permanent storage. The protocols for the collection of specimens for each of the various NBSB projects were developed in conjunction with individuals involved in sampling of these specimens to achieve a suitable procedure within the bounds of practicality. As part of the sampling protocol, information describing the sample and the sampling site is recorded; this information is maintained, both in hard copy and in a computer database, as part of the documentation for each sample in the specimen bank. For example, in the human liver project the following information concerning the donor is recorded (if available): age, sex, race, height, weight, cause of death, residence, occupational history, and diseases. For the marine mammal samples, information is recorded describing both the animal and the collection site. Information on the animal includes species, sex, age, various size measurements, stomach contents, reproductive status, health status, and general appearance. Information on the site and collection includes sample source (stranded, subsistence hunt, or incidental catch), method of collection, latitude/longitude, and weather conditions. If there are any deviations from the prescribed collection protocol, these changes must be noted on the sampling form. If additional tissue samples are taken from the same animals for use by other researchers, the samples and researchers are noted on the forms. In addition to the information concerning the animal or donor, the complete handling of the sample is documented, i.e., date and time of sampling, processing, and freezing; temporary storage conditions (if any); and when the sample is shipped to and received at the NBSB facility.

**Procedures and Materials (e.g., Sampling Implements and Containers) Should Be Selected to Minimize Potential Contamination and Degradation of the Sample.** Since the purpose of a specimen bank is the long-term preservation of specimens that are representative of the state of a site or organism immediately prior to collection, a major concern of specimen banking efforts is that the samples be collected, processed, and stored under conditions that minimize contamination of the specimen or any other changes in their chemical composition. Only selected materials are used for contact with the sample during collection and

storage to minimize potential sample contamination. For example, when samples require dissection (e.g., in the case of human liver, fish tissues, or marine mammal tissues), a titanium-bladed knife is used to avoid contamination from environmentally important trace elements (e.g., Ni, Cr, and Fe) found in conventional cutting instruments. During sample preparation, contact with the specimen is generally limited to clean, dust-free Teflon surfaces and the specimens are stored in precleaned Teflon bags or jars. After the specimens are placed in the storage containers, they are frozen in liquid nitrogen as soon as feasible and transported frozen to the specimen bank facility at NIST, where the samples are stored in liquid nitrogen vapor freezers at  $-150^{\circ}\text{C}$ .

**Sample Processing in "Clean" Environment.** To minimize contamination of the specimens during processing in the laboratory, specimen banking facilities should include "clean" laboratories. The design of the NBSB facility, which contains both class 100 sample processing areas and a class 1000 storage room, has been described previously (17).

**Cryogenic Homogenization.** The preparation of homogeneous subsamples for analysis from the bulk sample is a major requirement for specimen banking. Identical subsamples (i.e., homogeneous) are necessary to allow for valid comparison of data obtained by various researchers and different analytical techniques and for evaluation of the stability of specimens during storage. To address this requirement, a cryogenic homogenization procedure using Teflon disk mills was developed for NBSB samples (18). These mills are capable of homogenizing 50- to 700-g samples to provide homogeneous frozen samples with greater than 90% of the particles less than 0.46 mm in diameter and with subsampling errors due to inhomogeneity estimated at less than 2%. The use of the Teflon mills minimizes trace-element contamination that may occur with metal grinding/homogenization procedures. In addition, the low temperature grinding/homogenization reduces potential losses of volatile compounds and avoids thawing and refreezing of the specimen, thereby avoiding changes that could occur in the sample during these processes (e.g., degradation or rupture of tissue cells). Cryogenic homogenization has been used successfully at NIST for the homogenization of a variety of specimen types including: human liver, adipose tissue, and hair; mussel, oyster, and fish tissues (liver and muscle); honey bees; marine mammal tissues (liver, kidney, muscle, and

blubber); chicken tissue; and total human diet composites. This cryogenic homogenization procedure was used for the preparation of the first frozen tissue standard reference material issued at NIST, i.e., SRM 1974, Organics in Mussel Tissue (*Mytilus edulis*) (19).

### **Analysis of Selected Specimens within the Specimen Bank Program**

**A Formal Program Should Be Implemented for the Analysis of Archived Specimens.** Typically, all samples in a specimen bank would not be analyzed since this would be the function of a monitoring program. However, selected specimens should be routinely analyzed to provide data for the following purposes: to evaluate stability of analytes of interest and sample degradation during storage, to compare with results from samples collected in the future for long-term trend monitoring, and to compare with analytical results from other laboratories on samples collected at the same time for monitoring purposes.

**Analytical Group(s) Should Be Directly Associated with the Bank.** Because of the importance of analytical results obtained from banked specimens, a specimen bank program should be closely associated with a strong analytical group. Having an analytical group associated with the bank will provide a strong commitment to consistent, high-quality analytical results. In addition, there will be close interactions between the scientists involved in the selection of specimens and the development of collection protocols and those who will ultimately analyze the samples, thereby minimizing potential problems such as collection of insufficient sample for the required analyses or contamination of the sample during collection.

**Specimen Banks Serve an Important Quality Assurance (QA) Function for the Monitoring Program.** In the NS&T and NMMTB/AMMTAP specimen banking projects, other laboratories are responsible for the monitoring analyses. Thus, the analysis of selected specimens from the bank by NIST provides data for comparison with results from samples collected at the same time and analyzed as part of the associated monitoring program. These analyses then serve a quality-assurance function for the monitoring program.

### **Storage Conditions**

**Specimen Storage at  $-80^{\circ}\text{C}$  or Lower Is Recommended, Preferably in Liquid Nitrogen Vapor ( $-150^{\circ}\text{C}$ ).** One of the goals of the U.S. EPA human liver project

was to compare the stability of the analytes of interest under different storage conditions ( $-25$ ,  $-80$ , and  $-150^{\circ}\text{C}$ ). In a comparison of analyses of subsamples of human liver specimens stored at  $-25^{\circ}\text{C}$  and  $-150^{\circ}\text{C}$  for 7 years, no differences were for observed measurements of trace elements, PCB congeners, and chlorinated pesticides (3,4). However, even though the chemical analyses of the samples stored at the  $-25^{\circ}\text{C}$  indicated no significant changes in the content of the analytes measured, there was physical evidence of changes in the sample aliquots. At  $-25^{\circ}\text{C}$  the aliquots of frozen liver homogenate had formed ice crystals under the container lids and on the sample surface (i.e., the moisture in the samples had separated), and the homogenates were no longer powdery, but clumped. The samples stored at  $-150^{\circ}\text{C}$  were still powdery, as they had been at the time of homogenization. The subsample weights were stable over the 7-year storage interval for both storage conditions. However, the separation of moisture from the samples stored at  $-25^{\circ}\text{C}$  would necessitate the use of the total subsample for any analytical determinations. In addition, the color of the sample aliquots stored at  $-25^{\circ}\text{C}$  was different from those stored at  $-150^{\circ}\text{C}$ . Samples stored at  $-80^{\circ}\text{C}$  did not show any formation of ice crystals or any noticeable color changes. At present all specimens archived as part of NBSB projects are stored in liquid nitrogen vapor freezers at  $-150^{\circ}\text{C}$  to avoid the physical changes noted above. In addition, the liquid nitrogen vapor freezers are relatively maintenance free and low cost to operate compared to compressor-type freezers at  $-80$  or  $140^{\circ}\text{C}$ .

### **Specimen Access Policy**

Because of the costs associated with the collection and long-term storage of specimens and the increasing values of the specimens after years (even decades) of storage, a policy must be developed to determine access to portions of these specimens. However, before development of a suitable sample access policy, the following question must be addressed: What is the purpose of the specimen bank? Is the purpose of the bank to provide a bank of specimens that are available to all programs desiring specimens for monitoring purposes or a "long-term" collection of specimens for retrospective analyses that can only be accomplished with banked samples? Generally, the latter statement is true for most specimen banking programs. Thus, a specimen access policy should be established to address the disposition of the

banked tissues and the procedures, justification, and review process for tissue access. Requests for samples from the bank should be considered based on several criteria. First, requests should justify the need for use of banked tissue samples, i.e., is a banked specimen required to accomplish the goal or could specimens collected now serve the project? Second, QA procedures must be in place by those requesting the specimens to assure the quality of the analytical results obtained from the banked specimens. Finally, the requestor must agree to provide all analytical results to the specimen bank program for inclusion in the sample documentation.

Within the NBSB a formal specimen access policy has been developed for the two marine mammal projects. The basic philosophy of this access policy was first developed for the AMMTAP (10,11) and was then formalized for the NMMTB project (15). The basic points of this access policy are described briefly below. Duplicate samples (denoted A and B) of approximately 150 g for each tissue specimen are banked in the NMMTB. Subsamples of the "B" samples can be homogenized and aliquoted into approximately 20 subsamples of 6 to 8 g each, which are then available for scientific research. The "A" samples will remain as bulk samples and will only be homogenized after all portions from the "B" sample are depleted and sufficient justification exists to homogenize the remaining material. Thus, 50% of each specimen is available to the scientific community for research and scientific evaluations consistent with the goals of the NMMTB program and 50% is intended for long-term storage as a more permanent archive for posterity.

The homogenized subsamples of "B" samples are divided into three categories. Category 1 constitutes 40% of the homogenized material and is available to the scientific community for research that is consistent with the goals of the NMMTB. Formal requests for access to these tissues must be made as described below. Category 2 consists of 50% of the homogenized material and is reserved for use by the contributing agencies (NMFS is currently the only contributing agency). A procedure for access to these samples will be established by the contributing agencies and the NMMTB. Category 3 is the remaining 10% of the homogenized material, which is reserved to perform baseline analyses of the specimens to monitor storage stability, to compare with analyses from real-time monitoring programs, and to conduct research investigations within the NMMTB program.

If the "A" sample is eventually homogenized, the subsamples are divided into the following four categories. Category 1 consists of 25% of the material and is available to the scientific community as described above. Category 2 consists of 25% of the material and is reserved for use by the contributing agencies. Category 3 consists of 10% of the material for use within the NMMTB program as described above. Category 4 is the remaining 40% which is intended as a permanent archive that will not be utilized unless high priority needs exist; the determination of this need will be made by an advisory committee to the NMMTB. Combining the "A" and "B" samples, the total percentage sample allocations are as follows: Category 1, 32.5%; Category 2, 37.5%; Category 3, 10%; Category 4, 20%.

Formal requests for banked tissues in Category 1 must be submitted to the sponsor of the NMMTB and the request is reviewed by an informal review committee consisting of no less than three individuals, who are members of the NMMTB's advisory committee. Of particular importance in determining whether a sample request will be granted is the justification that samples from the NMMTB are required to accomplish the research and that suitable samples to accomplish the goals of the proposed research could not be obtained from other sources. The NMMTB is not intended to be used as a readily accessible source of marine mammal tissue for any researcher needing specimens, but only for research requiring banked samples from the past. This is an important point in particular for marine mammal tissues because of the restrictions associated with the acquisition of such tissues, since some of these animals are considered to be endangered species. In the banking of human tissue specimens, an access policy will be important because of legal considerations concerning privacy issues of the individual donors.

## Conclusions

The addition of a specimen banking component to an environmental monitoring program offers a number of advantages. However, to achieve the maximum benefit from these banked specimens, the program must be carefully designed. The NBSB has provided valuable experience in various aspects of specimen banking to assist in the design of future specimen banking projects.

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